ELUCIDATION OF A TRIPARTITE MECHANISM UNDERLYING THE IMPROVEMENT IN CARDIAC TOLERANCE TO ISCHEMIA BY COENZYME Q₁₀ PRETREATMENT

Coenzyme Q₁₀, which is involved in mitochondrial adenosine triphosphate production, is also a powerful antioxidant. We hypothesize that coenzyme Q₁₀ pretreatment protects myocardium from ischemia reperfusion injury both by its ability to increase aerobic energy production and by protecting creatine kinase from oxidative inactivation during reperfusion. Isolated hearts (six per group) from rats pretreated with either coenzyme Q₁₀, 20 mg/kg intramuscularly and 10 mg/kg intraperitoneally (treatment) or vehicle only (control) 24 and 2 hours before the experiment were subjected to 15 minutes of equilibration, 25 minutes of ischemia, and 40 minutes of reperfusion. Developed pressure, contractility, compliance, myocardial oxygen consumption, and myocardial aerobic efficiency were measured. Phosphorus 31 nuclear magnetic resonance (³¹P-NMR) spectroscopy was used to determine adenosine triphosphate and phosphocreatine concentrations as a percentage of a methylene diphosphonic acid standard. Hearts were assayed for myocardial coenzyme Q₁₀ and myocardial creatine kinase activity at end equilibration and at reperfusion. Treated hearts showed higher myocardial coenzyme Q₁₀ levels ($133 \pm 5 \mu\text{g/gm}$ ventricle versus $117 \pm 4 \mu\text{g/gm}$ ventricle, $p < 0.05$). Developed pressure at end reperfusion was $62\% \pm 2\%$ of equilibration in treatment group versus $37\% \pm 2\%$ in control group, $p < 0.005$. Preischemic myocardial aerobic efficiency was preserved during reperfusion in treatment group ($0.84 \pm 0.08 \text{ mm Hg}/(\mu\text{l O}_2/\text{min/gm ventricle})$ vs $1.00 \pm 0.08 \text{ mm Hg}/(\mu\text{l O}_2/\text{min/gm ventricle})$ at equilibration, $p = \text{not significant}$), whereas in the control group it fell to $0.62 \pm 0.07 \text{ mm Hg}/(\mu\text{l O}_2/\text{min/gm ventricle})$, $p < 0.05$ vs equilibration and vs the treatment group at reperfusion. Treated hearts showed higher adenosine triphosphate and phosphocreatine levels during both equilibration (adenosine triphosphate $49\% \pm 2\%$ for the treatment group vs $33\% \pm 3\%$ in the control group, $p < 0.005$; phosphocreatine $49\% \pm 3\%$ in the treatment group vs $35\% \pm 3\%$ in the control group, $p < 0.005$) and reperfusion (adenosine triphosphate $18\% \pm 3\%$ in the treatment group vs $11\% \pm 2\%$ in the control group, CTRL $p < 0.05$; phosphocreatine $45\% \pm 2\%$ in the treatment group vs $23\% \pm 3\%$ in the control group, $p < 0.005$). Creatine kinase activity in treated hearts at end reperfusion was $74\% \pm 3\%$ of equilibration activity vs $65\% \pm 2\%$ in the control group, $p < 0.05$). Coenzyme Q₁₀ pretreatment improves myocardial function after ischemia and reperfusion. This results from a tripartite effect: (1) higher concentration of adenosine triphosphate and phosphocreatine, initially and during reperfusion, (2) improved myocardial aerobic efficiency during reperfusion, and (3) protection of creatine kinase from oxidative inactivation during reperfusion. (J THORAC CARDIOVASC SURG 1996;111:443-50)

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Myocardial ischemia-reperfusion injury is associated with toxic oxygen metabolite production that causes lipid and protein peroxidation and energetic derangements as a result of alterations in energy synthesis, transfer, or use.¹⁻⁵ Oxygen radical scavengers and agents that modulate energy use and production have been used to prevent or attenuate this injury.⁶⁻¹⁰ Coenzyme Q₁₀ (CoQ₁₀) has both of these properties.¹¹

CoQ₁₀ is a lipid-soluble benzoquinone that has properties potentially useful in preventing or attenuating the damage associated with ischemia and reperfusion.^{11,12} CoQ₁₀ is directly involved in energy transduction and aerobic adenosine triphosphate (ATP) production; it transports electrons in the respiratory chain and couples the respiratory chain to oxidative phosphorylation.¹³⁻¹⁶ CoQ₁₀ is a regenerable and powerful antioxidant, capable of protecting cell structures from oxidative damage during reperfusion.¹⁷⁻¹⁹ It also regenerates vitamin E from the tocopheroxyl radical produced by free oxygen radicals.¹⁷ The antioxidant actions of CoQ₁₀ are not limited to the mitochondria but are applicable to any other cell membrane containing CoQ₁₀.¹⁷ These properties make CoQ₁₀ an ideal therapeutic agent to reduce myocardial ischemia-reperfusion injury.²⁰⁻²²

Creatine kinase (CK) is a crucial enzyme involved in the metabolism of high-energy phosphates. CK is exquisitely sensitive to oxidation and has been shown to be susceptible to inactivation by toxic oxygen metabolites during reperfusion, impairing the transfer of high-energy phosphates between phosphocreatine and adenosine diphosphate.^{4,5,23,24} We hypothesized that CoQ₁₀ pretreatment protects myocardium from ischemia reperfusion injury by increasing aerobic energy production and by protecting CK from oxidative inactivation during reperfusion.

Method

Pretreatment with CoQ₁₀. Male Sprague-Dawley rats weighing 200 to 250 gm (six per group) were divided into two groups. The CoQ₁₀ group was pretreated with CoQ₁₀ (20 mg/kg intramuscularly and 10 mg/kg intraperitoneally; Pharma Nord, Vejens, Denmark), control group was pretreated with vehicle only (dimethyl sulfoxide, Sigma Chemical Co., St. Louis, Mo.); both groups underwent pretreatment 24 hours and 2 hours before the experiment.

Isolated heart preparation. Rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally) and heparin sodium (500 IU intraperitoneally) in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research

and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). Hearts were excised quickly and arrested in a 4° C Krebs-Henseleit solution of the following composition: sodium chloride, 118 mmol/L; potassium chloride, 4.6 mmol/L; potassium phosphate, 1.17 mmol/L; magnesium sulfate, 1.17 mmol/L; calcium chloride, 1.16 mmol/L; sodium bicarbonate, 23 mmol/L; and glucose, 5.3 mmol/L. The hearts were then transferred to a nonrecirculating Langendorff apparatus and perfused at a constant aortic pressure of 76 mm Hg at 37° C with Krebs-Henseleit solution (pH 7.4, oxygen tension > 500 mm Hg), previously equilibrated with a gas mixture of 92.5% oxygen and 7.5% carbon dioxide. Hearts were paced at 360 beats/min at two times threshold (Grass Instrument Co., Quincy, Mass.). An intraventricular latex balloon was inserted in the left ventricle through the mitral valve and attached to a pressure transducer (COBE Laboratories, Inc., Lakewood, Colo.). Left ventricular end-diastolic pressure was set at 6 mm Hg. Developed pressure (DP; peak systolic pressure minus end-diastolic pressure), contractility (+dP/dt), and compliance (−dP/dt) were recorded throughout the experiment. Coronary flow was measured by a digital flow meter (Humonics, Rancho Cordova, Calif.) by measuring pulmonary artery effluent. Data were amplified, acquired, and recorded on a Macintosh IIfx computer (Apple Computer, Inc., Cupertino, Calif.) with LabView software (National Instruments, Austin, Texas).

Experimental protocol. Hearts were subjected to 15 minutes of equilibration, 25 minutes of global normothermic ischemia, and 40 minutes of reperfusion. Ischemia was achieved by means of a stopcock at the level of the aortic root. Pacing was halted during ischemia, and heart temperature was maintained at 37° C by immersion in a water-jacketed, nongassed perfusate bath.

Determination of myocardial oxygen consumption (MVO₂) and myocardial aerobic efficiency (DP/MVO₂). At end-equilibration and at end-reperfusion, oxygen tension was determined with a blood gas analyzer (Corning-170 pH/Blood Gas Analyzer; Corning Inc., Medfield, Mass.) from perfusate samples drawn from the aortic cannula and from the coronary sinus. MVO₂ (μl O₂/min/gm ventricle, wet weight) was determined from the following formula:

$$\text{MVO}_2 = (\text{Solubility of oxygen at } 37^\circ \text{C}) \times (p_{a\text{O}_2} - p_{cs\text{O}_2}) \times CF / \text{Wet weight of ventricle.}$$

where $p_{a\text{O}_2}$ represents oxygen tension determined from perfusate drawn from the aortic cannula, $p_{cs\text{O}_2}$ represents oxygen tension in perfusate drawn from the coronary sinus, and CF represents coronary flow. DP/MVO₂ was defined as the ratio between DP (in millimeters of mercury) and MVO₂.

Determination of CK activity in heart tissue. Control and CoQ₁₀ hearts (six per group) were perfused for 15 minutes to determine baseline (preischemic) CK activity. After ischemia at the end of the 40 minutes of reperfusion, left and right ventricles were freeze clamped in liquid nitrogen and stored at −70° C. Tissue samples were

Fig. 1. Recovery of mechanical function during reperfusion expressed as a percentage of end-equilibration left ventricular DP. Asterisk indicates $p < 0.005$ versus control. Note improvement afforded by CoQ₁₀ pretreatment.

weighed, placed in 10 volumes/weight of cold isolation buffer (5 mmol/L potassium phosphate, 0.3 mol/L sucrose, 5 mmol/L morpholino propanesulfonic acid and 0.2 mmol/L ethylenediaminetetraacetic acid, pH 7.4), and homogenized at 4° C during 20 seconds with a Tekmar Tissumizer (Tekmar Co., Cincinnati, Ohio). Homogenates were centrifuged at 0° C for 10 minutes at 2000g. The supernatant was diluted to less than 1000 IU/L before assay of CK activity. The assay was performed with Sigma diagnostic kit no. 47-UV (Sigma Chemical Co.) on a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) in quartz cuvettes maintained at 30° C. Values were expressed as international units per gram of ventricle.

Determination of preischemic CoQ₁₀ concentrations in heart tissue. Control and CoQ₁₀ hearts (six per group) were perfused for 15 minutes. Hearts were freeze clamped in liquid nitrogen and stored at -70° C. CoQ₁₀ levels were determined according to the procedure described by Edlund.²⁵ Briefly, tissue samples were homogenized partly enzymatically (with collagenase in ultrabath) and partly mechanically. After extraction with 1-propanol and the addition of an internal standard (coenzyme Q₁₁), the residue was subjected to high-performance liquid chromatography with electrochemical detection, yielding the total tissue content of both CoQ₁₀ and coenzyme Q₉. Levels are expressed as micrograms per gram of dry ventricle.

Evaluation of high-energy phosphate metabolites. Parallel groups of hearts (six per group) were perfused according to the previously described protocol within a 11.5 Tesla Bruker AM 500 spectrometer (Bruker Medical Instruments, Inc., Billerica, Mass.) and phosphorus 31 nuclear magnetic resonance (³¹P-NMR) spectra were acquired. A capillary tube with a standard concentration of methylene diphosphonic acid (MDP) was placed next to the heart inside the NMR tube to act as an absolute standard. ³¹P-NMR spectra were obtained every 10 minutes with 416 45-degree angle pulses with an interpulse delay of 1 second. A line broadening of 15 Hz was used.

Inorganic phosphate, phosphocreatine, ATP, and MDP areas were determined by triangulation. To correct for partial saturation, fully relaxed ³¹P-NMR spectra were obtained at a relaxation delay of 15 seconds, and correction factors for inorganic phosphate, ATP, phosphocreatine, and MDP were determined. Only the β-ATP peak was used for ATP determination because the α and γ peaks contain other phosphorylated nucleotide bases. Values of inorganic phosphate, ATP, and phosphocreatine are expressed as a percentage of the MDP standard as well as in terms of the percentage of their respective preischemic levels. Intracellular pH (pHi) was determined only during ischemia from the chemical shift of inorganic phosphate relative to phosphocreatine by applying the following equation: $pH_i = 6.77 - \log[(2.52/[d - 3.22]) - 1]$,^{26, 27} where d is the chemical shift in parts per million. During equilibration and reperfusion the presence of two overlapping inorganic phosphate peaks (intracellular and buffer) made it impossible to determine the inorganic phosphate concentration and pHi.

Statistical analysis. Data are expressed as mean ± standard error of the mean. Paired and nonpaired t tests were used for statistical significance. Significance was assumed for $p < 0.05$.

Results

Mechanical function. Hearts perfused for 80 minutes of equilibration preserved $94\% \pm 2\%$ of their equilibration DP, justifying the stability of our model. Control and CoQ₁₀ hearts had the same DP at end-equilibration (109 ± 6 vs 107 ± 5 mmHg, $p =$ not significant [NS]). At end-reperfusion, CoQ₁₀ hearts recovered $62\% \pm 2\%$ of their preischemic DP whereas control hearts recovered only $37\% \pm 2\%$ ($p < 0.005$ vs control, Fig. 1.) Both +dP/dt and -dP/dt behaved in a similar fashion. At end-reper-

Fig. 2. DP/MVO₂ during equilibration and at end-reperfusion. During reperfusion CoQ₁₀ hearts maintained their preischemic efficiency, in contrast to control hearts. Asterisk indicates $p < 0.05$ versus CoQ₁₀ group at reperfusion and vs both groups at equilibration. V, Ventricle.

Fig. 3. Myocardial CK activity at end-equilibration and at end-reperfusion. Hearts pretreated with CoQ₁₀ maintained greater CK activity than did control hearts. Asterisk indicates $p < 0.05$ versus control at reperfusion. V, Ventricle.

fusion, CoQ₁₀ hearts had lower end-diastolic pressures than did control (40.8 ± 2 vs 56 ± 4 mm Hg, $p < 0.005$). The onset of contracture during ischemia (7.7 ± 0.4 vs 8.6 ± 0.4 minutes, $p = \text{NS}$) and its degree at end-ischemia (44.1 ± 2 vs 45 ± 1 mm Hg, $p = \text{NS}$) were the same in both groups.

DP/MVO₂. During equilibration, control and CoQ₁₀ hearts had the same DP/MVO₂ (1.09 ± 0.08 mm Hg/(μl O₂/min/gm ventricle) vs 1.00 ± 0.08 mm Hg/(μl O₂/min/gm ventricle). At end reperfusion, however, CoQ₁₀ hearts preserved their preischemic DP/MVO₂ (0.84 ± 0.08 mm Hg/(μl O₂/min/gm ventricle), $p = \text{NS}$ vs CoQ₁₀ at equilibration). Control DP/MVO₂, however, fell to 0.62 ± 0.07 mm Hg/(μl

O₂/min/gm ventricle), $p < 0.05$ vs control at equilibration and vs CoQ₁₀ at reperfusion (Fig. 2).

CK activity. Preischemic CK activity was unaffected by CoQ₁₀ pretreatment (590 ± 29 IU/gm ventricle vs 590 ± 26 IU/gm ventricle in control, $p = \text{NS}$). In control hearts, CK activity after 40 minutes of reperfusion fell to 390 ± 10 IU/gm ventricle. CoQ₁₀ hearts, however, lost less CK activity after reperfusion (435 ± 17 IU/gm ventricle, $p < 0.05$ vs control during reperfusion; (Fig. 3).

Preischemic myocardial CoQ₁₀ levels. Baseline myocardial CoQ₁₀ levels in CoQ₁₀ hearts averaged 133 ± 5 μg/gm dry ventricle. They averaged 117 ± 4 μg/gm dry ventricle in control hearts ($p < 0.05$).

Fig. 4. Phosphocreatine (PCr) levels during equilibration (EQ) and during reperfusion (RP). CoQ₁₀ hearts showed higher levels during both equilibration and reperfusion. Asterisk indicates $p < 0.005$ versus control. Horizontal axis indicates time in minutes.

Fig. 5. ATP levels during equilibration (EQ) and during reperfusion (RP). CoQ₁₀ hearts showed higher levels during both equilibration and reperfusion. Asterisk indicates $p < 0.05$ versus control. Horizontal axis indicates time in minutes.

³¹P-NMR. At end-equilibration, hearts pretreated with CoQ₁₀ showed higher phosphocreatine levels than did control ($49\% \pm 3\%$ vs $35\% \pm 3\%$ MDP, $p < 0.005$) as well as higher ATP levels ($49\% \pm 2\%$ vs $33\% \pm 3\%$ MDP, $p < 0.005$). These higher levels are maintained throughout reperfusion; by end-reperfusion, phosphocreatine levels in CoQ₁₀ hearts were $45\% \pm 2\%$ versus $23\% \pm 3\%$ MDP in control hearts ($p < 0.005$), and ATP levels were $18\% \pm 3\%$ versus $11\% \pm 2\%$ MDP in control hearts ($p < 0.05$; Fig. 4 and 5). At end-ischemia, CoQ₁₀ and control hearts showed similar ATP levels ($10.5\% \pm 3\%$ vs $6.8\% \pm 2.4\%$ MDP, $p = \text{NS}$) and similar pH_i (6.24 ± 0.15 vs 6.27 ± 0.15 , $p = \text{NS}$). Phosphocreatine and ATP levels during reperfusion

can be also expressed as a percentage of equilibration levels (phosphocreatine $92\% \pm 7\%$ in CoQ₁₀ vs $66\% \pm 8\%$ in control, $p < 0.05$; ATP $39\% \pm 7\%$ in CoQ₁₀ vs $32\% \pm 5\%$ in control, $p = \text{NS}$).

Discussion

This experiment shows that CoQ₁₀ pretreatment improves myocardial tolerance to ischemia reperfusion injury. Not only does CoQ₁₀ improve the recovery of DP, it also improves diastolic function during reperfusion, as seen by a lower $-dP/dt$ and a lower end-diastolic pressure.

Exogenously administered CoQ₁₀ has been shown to nonspecifically incorporate into cell and mitochondrial membranes.^{28, 29} We have shown an incre-

ment in myocardial CoQ₁₀ levels in rats pretreated with CoQ₁₀ compared with control rats. Although CoQ₁₀ synthesis decreases during ischemia,³⁰ human and animal studies have shown that CoQ₁₀ pretreatment sufficient to increase preischemic CoQ₁₀ levels, as in our experimental model, prevents the myocardial CoQ₁₀ depletion that normally occurs during ischemia and reperfusion.³⁰⁻³³

As a member of the respiratory chain, CoQ₁₀ is involved in the flux of electrons as well as in the coupling of the respiratory chain to oxidative phosphorylation.¹³⁻¹⁶ CoQ₁₀ controls the efficiency of oxidative phosphorylation.¹⁴ Exogenously administered CoQ₁₀ appears to be able to increase the concentrations of high-energy phosphates, as shown by ATP and phosphocreatine levels during equilibration. Although this increment in high-energy phosphate levels was not translated into better mechanical function before ischemia, during reperfusion CoQ₁₀ hearts showed not only higher phosphocreatine and ATP levels but also improved recovery of mechanical function. Although this increment in aerobic energy production compared with control hearts could be sufficient to explain the improvement in mechanical function during reperfusion, it is interesting that before ischemia the elevation in high-energy phosphate levels did not improve mechanical function. This apparent paradox is obviously not addressed by evaluation of high-energy phosphate levels alone. The increment in ATP and phosphocreatine levels seen during equilibration in CoQ₁₀ hearts may limit myocardial damage during ischemia by better preserving cell structure and function, although our experiments were not designed to test this supposition. The observation that end-ischemic ATP levels and onset of contracture were similar in both groups is puzzling and obviously requires further investigation.

Myocardial CK, is inactivated during reperfusion by toxic oxygen metabolites, and this inactivation is directly responsible for limiting recovery of mechanical function.^{4, 5, 24} This loss of myocardial CK activity during reperfusion is the result of oxidation of its thiol groups and does not result, as we have previously shown, from washout of CK from damaged cells.⁴ This inactivation is responsible at least in part for lower ATP levels as a result of the impaired transfer of high-energy phosphates between phosphocreatine and adenosine diphosphate. As a result, less ATP is available to sustain myofibrillar activity.⁴ CoQ₁₀ hearts showed higher CK activity as well as higher ATP levels during reperfusion, both of which

are consistent with improved CK activity during reperfusion provided by CoQ₁₀. Although the mechanism is not specifically addressed in this study, CK protection is almost certainly the result of CoQ₁₀ antioxidant activity because oxidation is the primary mechanism for reperfusion-induced inactivation. Another mechanism that may explain higher levels of myocardial CK activity during reperfusion in CoQ₁₀ hearts is the ability of CoQ₁₀ to stabilize cell membranes.¹¹ On the basis of the results of previous experiments in which we showed no significant increment of CK in the coronary effluent during reperfusion, we believe that cell membrane leakage is not responsible for loss of CK activity during reperfusion, and stabilization of cell membranes by CoQ₁₀ is therefore not the mechanism responsible for the protection of CK activity during reperfusion.

Finally, CoQ₁₀ pretreatment preserves DP/MVO₂ during reperfusion with respect to that in the control group. Hearts stunned by ischemia-reperfusion consume more oxygen to generate less pressure than during equilibration and are therefore less efficient.³⁴⁻³⁸ Several authors have shown that mitochondrial function is not the limiting factor in recovery of mechanical function in stunned myocardium.³⁷⁻⁴⁰ Rather, alterations in electromechanical coupling, ionic transport (calcium channels, ATP-dependent potassium channels), and muscle contraction are principally involved in the increased cost of contraction in the stunned myocardium with respect to MVO₂.⁴¹⁻⁴⁶ CoQ₁₀ may protect reperfused myocardium through its antioxidant and membrane-stabilizing properties, as well as through its ability to increase ATP levels to support energy-consuming processes, allowing DP/MVO₂ to remain at normal levels.

Conclusions

We conclude from these experiments that CoQ₁₀ pretreatment effectively increases myocardial CoQ₁₀ levels, leading to an improved tolerance to myocardial reperfusion injury. This improvement results from the effects of CoQ₁₀ on mitochondrial function and antioxidant activity. CoQ₁₀ increases myocardial energy production, protects CK during reperfusion, preserves DP/MVO₂, and improves mechanical function after ischemic insult.

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Discussion

Dr. Pedro J. del Nido (Boston, Mass.). I congratulate you on an elegant study and thank you for sending me the manuscript ahead of time.

This is an important mechanism that you have outlined here, and one that has been studied by several investigators in the past. All have also demonstrated that CoQ_{10} is very effective, with both experimental studies and, I may now add, human data showing that it may in fact be useful. The important aspect of your study is that you have convincingly demonstrated that the mechanism may in fact be its antioxidant protection of CK. One of the major effects of the presence of CK is to lower free ATP levels;

the free energy of ATP hydrolysis, which is required to drive the calcium pumps, is thus preserved.

You have available, however, a means of measuring the activity of CK *in vivo* by performing saturation transfer experiments with NMR and measuring the free energy of ATP hydrolysis. Have you done those experiments to corroborate the *in vitro* assays of CK enzyme activity?

Second, with respect to the question of whether the CoQ_{10} is actually used, have you looked at UBIQUINOL levels in these hearts after administration?

Dr. Crestanello. We have not looked at CK activity through saturation transfer or looked at the free energy of ATP hydrolysis in this animal model, but we have plans to do that.

Regarding your second question on the UBIQUINOL levels in the heart, we measured them. Those are the levels that we have shown as the levels of CoQ_{10} in the myocardium.

Dr. Michael A. Rowland (Melbourne, Australia). I commend you for tackling the difficult question of the mechanism of action of this important and interesting compound. The improved postischemic DP/MVO₂, higher ATP and phosphocreatine levels, and preservation of CK activity are all indicative of a myoprotective effect. You would expect to see these with other forms of protection, such as hypothermia or cardioplegia, and they are not specific to CoQ_{10} .

The mechanism of this effect probably relates to CoQ_{10} as a potent free radical scavenger, which brings me to my first question. Did you measure indexes of oxidative stress such as thiobarbituric acid-reactive substances or reduced oxidized glutathione ratio? If so, was there a difference between the two groups?

Second, I was interested to see the elevated levels of phosphocreatine and ATP in prestress measurements of the treated group. Does this represent a supernormal level of high-energy phosphates, or is this a reflection of the myoprotective effects of CoQ_{10} against the insult to the heart of the setting and preparation?

Finally, we believe that another protective action of CoQ_{10} may play an important role, particularly in the senescent myocardium. The unique position of CoQ_{10} as a mobile electron carrier within the mitochondrial membrane enables it to maintain the flow of energy and sustain ATP production in the face of both acute and chronic free radical injury to the mitochondrial membrane and the protein complexes. To what extent do you think that this mechanism could be operational in your model?

Dr. Crestanello. Regarding your first question, if we have looked at other ways to measure oxidative stress in our model, we have used lucigenin-enhanced chemiluminescence to continuously and directly measure free oxygen radical production. We have shown that hearts pretreated with CoQ_{10} had lower levels of oxidative stress than control hearts.

Regarding your second question about the higher levels of high-energy phosphates at equilibration before the ischemic insult, we attribute this effect of CoQ_{10} to the improvement in oxidative phosphorylation and respiratory chain efficiency. Regarding your last question, I agree that the last mechanism you outlined is very important, and it could also explain our results.